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## (54) Title: HYPERSENSITIVE RESPONSE ELICITOR-INDUCED STRESS RESISTANCE

## (57) Abstract

The present invention is directed to imparting stress resistance to plants. This can be achieved by applying a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart stress resistance to plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the elicitor can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart stress resistance to plants or plants grown from the plant seeds.

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## HYPERSENSITIVE RESPONSE ELICITOR-INDUCED STRESS RESISTANCE

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### FIELD OF THE INVENTION

10                   The present invention relates to imparting stress resistance to plants with a hypersensitive response elicitor.

### BACKGROUND OF THE INVENTION

15                   Under both natural and agricultural conditions, plants are exposed to various forms of environmental stress. Stress is mainly measured with respect to growth (i.e. biomass accumulation) or with respect to the primary assimilation processes (i.e. carbon dioxide and mineral intake). Soil water deficits, suboptimal and supraoptimal temperatures, salinity, and poor aeration of soils may each cause some 20 growth restrictions during the growing season, so that the yield of plants at the end of the season expresses only a small fraction of their genetic potential. Indeed, it is estimated that in the United States the yield of field-grown crops is only 22% of 25 genetic potential. The same physicochemical factors can become extreme in some habitats, such as deserts or marshes, and only specially adapted vegetation can complete its life cycle in the unusually hostile conditions. In less extreme environments, individual plants can become acclimated to changes in water potential, temperature, salinity, and oxygen deficiency so that their fitness for those environments improves. Some species are better able to adapt than others, and various anatomical, structural, and biochemical mechanisms account for acclimation.

30                   Under natural and agriculture conditions, plants must constantly endure stress. Some environmental factors can become stressful in a very short period of time (e.g., high or low temperature) or may take long periods of time to stress plants (e.g., soil water content or mineral nutrients). Generally, environmental stress effecting plants can be in the form of climate related stress, air pollution stress,

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chemical stress, and nutritional stress. Examples of climate related stress include drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light. Air pollution stress can be in the form of carbon dioxide, carbon monoxide, sulfur dioxide, NO<sub>x</sub>, hydrocarbons, ozone, ultraviolet radiation, and acidic rain. Chemical stress can result from application of insecticides, fungicides, herbicides, and heavy metals. Nutritional stress can be caused by fertilizers, micronutrients, and macronutrients.

For most plants, water is essential for growth. Some plants are able to preserve some water in the soil for later use, while others complete their life cycles during a wet season before the onset of any drought. Other plants are able to aggressively consume water to save themselves while causing water deprivation for other plants in that location. Plants lacking any of these capabilities are severely hampered by the absence of water.

Chilling injury occurs in sensitive species at temperatures that are too low for normal growth but not sufficiently low to form ice. Such injury typically occurs in species of tropical or subtropical origin. When chilling occurs, discoloration or lesions appear on leaves giving them a water-soaked appearance. If roots are chilled, the plants may wilt. On the other hand, freezing temperatures and the accompanying formation of ice crystals in plants can be lethal if ice crystals extend into protoplasts or remain for long periods.

Stress is also caused by the other temperature extremes with few plants being able to survive high temperatures. When higher plant cells or tissues are dehydrated or are not growing, they can survive higher temperatures than cells which are hydrated, vegetative, and growing. Tissues which are actively growing can rarely survive at temperatures above 45°C.

High salt concentrations are another form of environmental stress which can afflict plants. In natural conditions, such high concentrations of salt are found close to seashores and estuaries. Farther inland, natural salt may seep from geological deposits adjoining agricultural areas. In addition, salt can accumulate in irrigation water when pure water is evaporated or transpired from soil. About 10% of all irrigated farmland is effected by high salt concentrations. High salt content not

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only injures plants but degrades soil structure by decreasing porosity and water permeability.

Air pollution in the form of ozone, carbon dioxide, carbon monoxide, sulfur dioxide, NO<sub>x</sub>, and hydrocarbons can very adversely effect plant growth by  
5 creating smog and environmental warming.

The present invention is directed to overcoming various forms of environmental stress and imparting resistance in plants to such stress.

### SUMMARY OF THE INVENTION

10       The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart  
15 stress resistance. Alternatively, stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

20       Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO<sub>x</sub>, hydrocarbons, ozone, ultraviolet radiation,  
25 acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients). Applicants have found that use of hypersensitive response elicitors in accordance with the present invention impart resistance to plants against such forms of environmental stress.

### DETAILED DESCRIPTION OF THE INVENTION

30       The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one

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embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart stress resistance. Alternatively, the stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a 5 hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

The hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. 10 Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, 15 *Pseudomonas syringae*, *Pseudomonas solancearum*, *Xanthomonas campestris*, and mixtures thereof). In addition to hypersensitive response elicitors from these Gram negative bacteria, it is possible to use elicitors from Gram positive bacteria. One example is *Clavibacter michiganensis* subsp. *sepedonicus*.

An example of a fungal source of a hypersensitive response elicitor 20 protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from 25 *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser 1	5	10	15
Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser 20	25	30	
Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr 35	40	45	
Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu 50	55	60	

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	Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser	65	70	75	80
	Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys	85	90	95	
5	Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp	100	105	110	
	Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln	115	120	125	
10	Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met	130	135	140	
	Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly	145	150	155	160
	Asn <u>Gly</u> Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly	165	170	175	
15	Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu	180	185	190	
	Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala	195	200	205	
20	Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val	210	215	220	
	Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp	225	230	235	240
	Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp	245	250	255	
25	Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys	260	265	270	
	Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln	275	280	285	
30	Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr	290	295	300	
	Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala	305	310	315	320
	Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala	325	330	335	
35	Asn Ala				

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains

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substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

5	CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTGCA CACCGTTACG	60
	GCGTTTATGG CCGCGATGAA CCGGCATCAG CGGGCGCGCT GGTCGCCGCA ATCCGGCGTC	120
	GATCTGGTAT TTCAGTTGG GGACACCGGG CGTGAACTCA TGATGCAGAT TCAGCCGGGG	180
	CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG	240
	TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG	300
10	CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTGAACT GGCGGAAATG	360
	ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC	420
	CGATCATTAA GATAAAGGCG GCTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT	480
	CACCGTCGGC GTCACTCAGT AACAAAGTATC CATCATGATG CCTACATCGG GATGGCGTG	540
	GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	600
15	AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGCGTC TCCGGTCTGG GGCTGGGTGC	660
	TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT	720
	GAGCAGCACCC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGCGCGCT	780
	GGCGCAGGGG CTGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC	840
	TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGCGGCGA	900
20	TGCGTTGTCA AAAATGTTG ATAAAGCGCT GGACGATCTG CTGGGTCTG ACACCGTGAC	960
	CAAGCTGACT AACCAAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
	CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAAC GCACTGTCGT CCATTCTCGG	1080
	CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT	1140
	GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGCGT	1200
25	GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
	CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
	TCAGTATCCG GAAATATTCC GTAAACCGGA ATACCAAGAAA GATGGCTGGA GTTCGCCGAA	1380
	GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440
	CGCCAGCATG GACAAATTCC GTCAGGCAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1500
30	TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC	1560
	GGCTGTCGTC GGCGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACCCCTGATA	1620

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	ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTC	1680
	TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAACCA TCGTCATCGA TCTGGTACAA	1740
	ACGCACATTT TCCC GTT CAT TCGCGTCGTT ACGGCCACA ATCGCGATGG CATCTCCCTC	1800
	GTCGCTCAGA TTGCGCGGCT GATGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC	1860
5	CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTCTATCC GCCCTTTAG	1920
	CAGATAGATT GCGGTTCGT AATCAACATG GTAATGCGGT TCCGCCGTG CGCCGGCCGG	1980
	GATCACCAACA ATATTCAAG AAAGCTGTCT TGACACCTACC GTATCGCGG AGATACCGAC	2040
	AAAAATAGGGC AGTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG	2100
	GTTCGTCATC ATCTTTCTCC ATCTGGCGA CCTGATCGGT T	2141

10

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

15

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
 1 5 10 15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
 20 25 30

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Asn  
 20 35 40 45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
 20 35 40 45 50 55 60

Met Met Met Ser Met Met Gly Gly Gly Leu Met Gly Gly Gly Leu  
 20 35 40 45 50 55 60 65 70 75 80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 20 35 40 45 50 55 60 65 70 75 80 85 90 95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 20 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 20 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 20 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 20 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 20 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175

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	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu
					180					185				190		
	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly
					195			200			205					
5	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly
					210			215			220					
	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu
					225			230			235			240		
10	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln
					245			250			255					
	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln
					260			265			270					
	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe
					275			280			285					
15	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met
					290			295			300					
	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro
					305			310			315			320		
20	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser
					325			330			335					
	Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn
					340			345			350					
	Lys	Ala	Lys	Gly	Met	Ile	Lys	Arg	Pro	Met	Ala	Gly	Asp	Thr	Gly	Asn
					355			360			365					
25	Gly	Asn	Leu	Gln	Ala	Arg	Gly	Ala	Gly	Gly	Ser	Ser	Leu	Gly	Ile	Asp
					370			375			380					
	Ala	Met	Met	Ala	Gly	Asp	Ala	Ile	Asn	Asn	Met	Ala	Leu	Gly	Lys	Leu
					385			390			395			400		
	Gly	Ala	Ala													

30      This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from 35      *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,"

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Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

5	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTGAA TTATTCATAA GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTCT ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTGCCAGAA TGCTGGGTTG GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAAT ATGATACCGT CAATCAGCTG GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	60 120 180 240 300
10	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTTG GCTCAGGTGG CCTGGGCGAA GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTCGC TGAAACACGCT GGGCTCGAAA GGCGGCAACA ATACCACTTC AACAAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC CCGATGCAGC AGCTGCTGAA GATGTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	360 420 480 540 600
15	CAAGATGGCA CCCAGGGCAG TTCCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGT AATGCTGGCAC GGGTCTTGAC GGTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG TTAGGTAACG CCGTGGGTAC CCGTATCGGT ATGAAAGCGG GCATTCAAGGC GCTGAATGAT	660 720 780 840 900
20	ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTCGTCA ATAAAGGCGA TCGGGCGATG GCGAAGGAAA TCGGTCAAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAA AGCACTGAGC AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	960 1020 1080 1140 1200
25	GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1260 1288

Another potentially suitable hypersensitive response elicitor from  
30 *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927,  
which is hereby incorporated by reference. The protein is encoded by a DNA  
molecule having a nucleic acid sequence of SEQ. ID. No. 5 as follows:

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	ATGTCAATT C TTACGCTTAA CAACAATACC TCGTCCTCGC CGGGTCTGTT CCAGTCCGGG	60
5	GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT	120
	CGGCAAACCA TTGAGCAAAT GGCTCAATT A TTGGCGGAAC TGTTAAAGTC ACTGCTATCG	180
	CCACAATCAG GTAAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT	240
10	AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT	300
	CAGAACATGC TGAGTGAGAT GGGCAACAAAC GGGCTGGATC AGGCCATCAC GCCCCGATGGC	360
15	CAGGGCGGGCG GGCAGATCGG CGATAATCCT TTACTGAAAG CCATGCTGAA GCTTATTGCA	420
	CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC	480
	TCTTCCGGTA CTTCTTCATC TGGCGGTTCC CCTTTAACG ATCTATCAGG GGGGAAGGCC	540
20	CCTTCCGGCA ACTCCCCCTTC CGGCAACTAC TCTCCCGTCA GTACCTTCTC ACCCCCACCC	600
	ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTTCTT CTCCCACCAA AGCAGCCGGG	660
25	GGCAGCACGC CGGTAACCGA TCATCCTGAC CCTGTTGGTA GCGCGGGCAT CGGGGCCGGA	720
	AATTCCGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC	780
	GTGAAAGCGG GTCAGGTGTT TGATGGAAA GGACAAACCT TCACCGCCGG TTCAGAATTA	840
30	GGCGATGGCG GCCAGTCTGA AAACCAAGAAA CCGCTGTTA TACTGGAAGA CGGTGCCAGC	900
	CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC	960
35	AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC	1020
	AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC	1080
	AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC	1140
40	TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC	1200
	CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC	1260
	GTCAATACCA GTGATATCTC ACTGGGTGAT GTTAAAACCC ACTACAAAGT GCCGATGTCC	1320
45	GCCAACCTGA AGGTGGCTGA ATGA	1344

See GenBank Accession No. U94513. The isolated DNA molecule of the present  
 50 invention encodes a hypersensitive response elicitor protein or polypeptide having an  
 amino acid sequence of SEQ. ID. No. 6 as follows:

55	Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu	
	1 5 10 15	
	Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser	
	20 25 30	
60	Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala	
	35 40 45	

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Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly  
50 55 60

5 Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly  
65 70 75 80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro  
85 90 95

10 Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu  
100 105 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gln Ile Gly Asp  
15 115 120 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp  
130 135 140

20 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala  
145 150 155 160

Ser Ser Gly Thr Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
165 170 175

25 Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro  
180 185 190

30 Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro  
195 200 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
210 215 220

35 Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly  
225 230 235 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His  
245 250 255

40 Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln  
260 265 270

45 Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn  
275 280 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val  
290 295 300

50 Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala  
305 310 315 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr  
325 330 335

55 Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn  
340 345 350

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	Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
	355 360 365
5	Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
	370 375 380
	Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
	385 390 395 400
10	His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
	405 410 415
	Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
	420 425 430
15	Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
	435 440 445

20 This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from  
 25 *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 7 as follows:

30	ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCCAG TACACACAGC GGCGCACAAAC	60
	CCTGTGGGGC ATGGTGTTGC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC	120
	GCTGCATCAT TGGCCGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAACAT TCACCAGCCA	180
35	TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG	240
	GGCTGTTGG GGACGAAAAA ATTTTCAGA TCGGCACCGC AGGGCCAGCC AGGTACCAACC	300
40	CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT	360
	GAGGCAGCCCG CGCCAGATGC GGCGCGTTTG ACCCGTTCGG GCGGCGTCAA ACGCCGCAAT	420
	ATGGACGACA TGGCCGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA	480
45	ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC	540
	AAAATGGCTC ACCGGCTTC AGCCAACGCC GGCGATCGCC TGCAGCATTG ACCGCCGAC	600
50	ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA	660
	ACGGCCCCACG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA	720
	CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC	780
55	GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAAACTGA CTGCGGTTGC GGAAAGCGTC	840

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	CTTGAGGGGA CAGATACCAAC GCAGTCACCC CTTAACCGC AATCAATGCT GAAAGGAAGT	900
5	GGAGCCGGGG TAACGCCGCT GGCGGTAAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG	960
	GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC	1020
	TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
10	CACCTGTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
	GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA	1200
15	AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAAACAAT GCTAACCAA	1260
	CCGGGGGAAG CGCACCGTTC CTTATTAAACC GGCAATTGGC AGCATCCTGC TGGCGCAGCG	1320
	CGGCCGCAGG GCGAGTCAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG	1380
20	CTGGCGTAT GGCAATCTGC GGATAAAAGAT ACCCACAGCC AGCTGTCTCG CCAGGCAGAC	1440
	GCTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAATCC	1500
25	TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG	1560
	ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC	1620
	CCGGAGAGCC ATATTTCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC	1680
30	GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTTGTGGCC	1740
	GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG	1800
35	AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT	1860
	TCTGGATTTC TCCATGACGA CCACGGCCAG CTTAACGCGC TGGTAAAAAA TAACTTCAGG	1920
	CAGCAGCATG CCTGCCCGTT GGGTAACGAT CATCAGTTTC ACCCCGGCTG GAACCTGACT	1980
40	GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG	2040
	ATTCTTGATA TGGGGCATTG AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT	2100
45	GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAAGGC	2160
	CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCTGAA TATTAATCAG	2220
	AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTT CGCTGCCGCA TGTGCGCAAT	2280
50	AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAAG ACCATAAGGC CCAGGCCATG	2340
	GCGGTGATTC GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGCTCCTTC	2400
55	CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAACCTCTCAG CCGCGAAGGT	2460
	ATCAGCGGCG AACTGAAAGA CATTGATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC	2520
	CACGAGGGAG AGGTGTTCA TCAGCCGCGT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC	2580
60	AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC	2640
	CATGAGCACA AACCGATTGC CACCTTGTAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC	2700
65	GGCTGGCACG CCTATGCGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA	2760

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	CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG	2820
	TTGACGGTTA AGCTCTCGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC	2880
5	AGCAGTAAAT TTTCCGAAAG GATCCGCC TATGCGTTCA ACCCAACAAT GTCCACGCCG	2940
	CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGGC TGAGGGGTTG	3000
10	AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAAACAAC TGGATGCGCA TAACGTTCGT	3060
	CATAACCGCG CACAGCCAGA TTTGCAGAGC AAACCTGGAAA CTCTGGATT AGGCGAACAT	3120
	GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCCGACG AACTGGAGCA GAGTGCAACC	3180
15	CGTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT	3240
	AGCGAATTAA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT	3300
	GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG	3360
20	AGTAAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG	3420
	AAGGGCGAGA TCCCCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCAA	3480
25	TCGCGTTAA TTTTAGATAC CGTGACCATC GGTGAACACTGC ATGAACTGGC CGATAAGGCG	3540
	AAACCTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC	3600
	GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC	3660
30	TTCACCCATA ATAAGGCCT GGAAGCAAAC TATGATGCCG TCAAAGCCTT TATCAATGCC	3720
	TTTAAGAAAG AGCACCCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCACAGGGC	3780
35	AGTGC GGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAGT	3840
	ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCTTAGC	3900
	AAGAAGGTGC CAGTTCCGGT GATCCCCGA GCCGGCATCA CGCTGGATCG CGCCTATAAC	3960
40	CTGAGCTTCA GTCGTACCAAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGGGGGTG	4020
	AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA	4080
45	ACCAGTGCAG GTAACGCCAG TGAACGGTTG AGCGCAAAAC ATAAAATCAG CCCGGACTTG	4140
	CGTATCGCG CTGCTGTGAG TGGCACCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT	4200
	AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC	4260
50	CCGGCAGAAC TGTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG	4320
	TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGGCG GTATCAATCT GAACGAAGAC	4380
55	GGCAGTAAAC CAAATGGTGT CACTGCCGT GTTCTGGCG GGCTAAGTGC ATCGGCAAAAC	4440
	CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG	4500
	GCCAGCAATA ACCGCCAAC CTTCCCTAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT	4560
60	GCTTTAGGGG TTGCCATTCACTACGCAT GAAGGGAAAC CGGTCGGGAT CTTCCCGGCA	4620
	TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC	4680
65	AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC	4740



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	Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro	
	145 150 155 160	
5	Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln	
	165 170 175	
	Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp	
	180 185 190	
10	Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile	
	195 200 205	
	Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala	
	210 215 220	
15	Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln	
	225 230 235 240	
20	Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro	
	245 250 255	
	Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys	
	260 265 270	
25	Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln	
	275 280 285	
	Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val	
30	290 295 300	
	Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro	
	305 310 315 320	
35	Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys	
	325 330 335	
	Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln	
	340 345 350	
40	His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr	
	355 360 365	
	Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys	
45	370 375 380	
	Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys	
	385 390 395 400	
50	Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr	
	405 410 415	
	Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile	
	420 425 430	
55	Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg	
	435 440 445	
	Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp	
60	450 455 460	
	Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp	
	465 470 475 480	
65	Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser	
	485 490 495	

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Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser  
 500 505 510  
 Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg  
 515 520 525  
 5 His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His  
 530 535 540  
 10 Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His  
 545 550 555  
 Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg  
 565 570 575  
 15 Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro  
 580 585 590  
 Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His  
 595 600 605  
 20 Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe  
 610 615 620  
 His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg  
 625 630 635 640  
 Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly  
 645 650 655  
 30 Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His  
 660 665 670  
 His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly  
 675 680 685  
 35 Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr  
 690 695 700  
 Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly  
 705 710 715 720  
 40 Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu  
 725 730 735  
 Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val  
 740 745 750  
 45 Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu  
 755 760 765  
 Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly  
 770 775 780  
 50 Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe  
 785 790 795 800  
 Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu  
 805 810 815  
 55 Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His  
 820 825 830  
 Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln  
 835 840 845  
 60 65

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	Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys			
	850	855	860	
5	Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser			
	865	870	875	880
	His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln			
	885	890	895	
10	Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro			
	900	905	910	
	Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met			
	915	920	925	
15	Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys			
	930	935	940	
20	Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val			
	945	950	955	960
	Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr			
	965	970	975	
25	Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His			
	980	985	990	
	Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly			
	995	1000	1005	
30	Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro			
	1010	1015	1020	
35	Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His			
	1025	1030	1035	1040
	Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu			
	1045	1050	1055	
40	Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val			
	1060	1065	1070	
	Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly			
	1075	1080	1085	
45	Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu			
	1090	1095	1100	
50	Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu			
	1105	1110	1115	1120
	Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp			
	1125	1130	1135	
55	Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro			
	1140	1145	1150	
	Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val			
	1155	1160	1165	
60	Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser			
	1170	1175	1180	
65	Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe			
	1185	1190	1195	1200

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	Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr			
	1205	1210	1215	
5	Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp			
	1220	1225	1230	
	Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val			
	1235	1240	1245	
10	Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu			
	1250	1255	1260	
15	Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser			
	1265	1270	1275	1280
	Met Ser Phe Ser Arg Ser Tyr Gly Gly Val Ser Thr Val Phe Val			
	1285	1290	1295	
20	Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly			
	1300	1305	1310	
	Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly			
	1315	1320	1325	
25	Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile			
	1330	1335	1340	
30	Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys			
	1345	1350	1355	1360
	Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile			
	1365	1370	1375	
35	Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly			
	1380	1385	1390	
	Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro			
	1395	1400	1405	
40	Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu			
	1410	1415	1420	
45	Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr			
	1425	1430	1435	1440
	Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn			
	1445	1450	1455	
50	Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser			
	1460	1465	1470	
	Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg			
	1475	1480	1485	
55	Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn			
	1490	1495	1500	
60	Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala			
	1505	1510	1515	1520
	Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly			
	1525	1530	1535	

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	Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
	1540 1545 1550
5	Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
	1555 1560 1565
	Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
	1570 1575 1580
10	His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
	1585 1590 1595 1600
	Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
	1605 1610 1615
15	Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
	1620 1625 1630
20	Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
	1635 1640 1645
	Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
	1650 1655 1660
25	Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
	1665 1670 1675 1680
	Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
	1685 1690 1695
30	Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
	1700 1705 1710
35	Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
	1715 1720 1725
	Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
	1730 1735 1740
40	Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
	1745 1750 1755 1760
	Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
	1765 1770 1775
45	Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
	1780 1785 1790
50	Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
	1795 1800 1805
	Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
	1810 1815 1820
55	Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
	1825 1830 1835

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

60 The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 9 as follows:

ATGACATCGT CACAGCAGCG GGTTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTAAA 60  
ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAAGA TGAGGAGGCG 120  
5 GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG 180  
GCTGACCCAC AAACCTCAAT AACCCCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG 240  
10 GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACCTGC ACAACGTGCG TTTATGTTT 300  
CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTAGCG ATATCGTTAG CGGCTTCATC 360  
15 GAACATGCGG CAGAAGTGCAG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA 420

This is known as the *dspF* gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ ID No. 10 as follows:

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

50 The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

55 Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
1 5 10 15

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	Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser			
	20	25	30	
	Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met			
5	35	40	45	
	Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala			
	50	55	60	
	Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Ile Glu Asp Val			
	65	70	75	80
10	Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe			
	85	90	95	
	Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met			
	100	105	110	
15	Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu			
	115	120	125	
	Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met			
	130	135	140	
	Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro			
	145	150	155	160
20	Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe			
	165	170	175	
	Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile			
	180	185	190	
25	Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly			
	195	200	205	
	Thr Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser			
	210	215	220	
	Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser			
	225	230	235	240
30	Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp			
	245	250	255	
	Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Leu Gly Thr Pro Val			
	260	265	270	
	Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln			
35	275	280	285	
	Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala			
	290	295	300	
	Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala			
	305	310	315	320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
325 330 335

Asn Gln Ala Ala Ala  
340

- 5 This hypersensitive response elicitor polypeptide or protein has a molecular weight of  
34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.  
Further information about the hypersensitive response elicitor derived from  
*Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer,  
10 “*Pseudomonas syringae* pv. *syringae* Harpin<sub>PsS</sub>: a Protein that is Secreted via the Hrp  
Pathway and Elicits the Hypersensitive Response in Plants,” *Cell* 73:1255-1266  
(1993), which is hereby incorporated by reference. The DNA molecule encoding the  
hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide  
sequence corresponding to SEQ. ID. No. 12 as follows:

15	ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCCCTG GTACGTCCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCCTTCA GGAAGTTGTC GTGAAGCTGG CCGAGGAACG GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA AAACTGTTGG CCAAGTCGAT GGCGCAGAT GGCAAGGCAG GCGGCGGTAT TGAGGATGTC	60 120 180 240
20	ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAGGAAG ACAACTTCCT TGATGGCGAC	300 360 420 480 540
25	GAAACGGCTG CGTTCCGTTG GGCACACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTCC AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC GGCAATAACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA	600 660 720 780
30	TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCCGAGAC CGGTACGTGCG GCGAATGGCG GACAGTCCGC TCAGGGATCTT GATCAGTTGC TGGCGGGCTT GCTGCTCAAG GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT	840 900 960
	GCGCAAATCG CCACCTTGCT GGTCAGTAGC CTGCTGCAAG GCACCCGCAA TCAGGCTGCA GCCTGAA	1020 1026

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference. The protein has a nucleotide sequence of 5 SEQ. ID. No. 13 as follows:

	TCCACTTCGC	TGATTTGAA	ATTGGCAGAT	TCATAGAAC	GTTCAAGGTGT	GGAAATCAGG	60		
10	CTGAGTGC	GC AGATTCGTT	GATAAGGGTG	TGGTACTGGT	CATTGTTGGT	CATTTCAAGG	120		
	CCTCTGAGTG	CGGTGCGGAG	CAATACCA	GT CTTCTGCTG	GCGTGTGCAC	ACTGAGTCGC	180		
	AGGCATAGGC	ATTTCA	TTTCAGTTC	CTTGC	GTGTTGG	TTGGGCATAT	AAAAAAAGGA	ACTTTAAAAA	240
15	ACAGTGCA	AT GAGATGCCGG	CAAAACGGGA	ACCGGTCGCT	GCGCTTTGCC	ACTCA	TTTCG	300	
	AGCAAGCTCA	ACCCC	AAACA	TCCACATCCC	TATCGAACGG	ACAGCGATAC	GGCCACTTGC	360	
20	TCTGGTAAAC	CCTGGAGCTG	GC	GTGCGGTCC	AATTGCCCAC	TTAGCGAGGT	AA	CGCAGCAT	420
	GAGCATCGGC	ATCACACCCC	GGCGCAACA	GACCACCA	CG	CACTCGATT	TTT	CGCGC	480
	AAGCGGCAAG	AGT	CCTCAAC	CAAACACGTT	CGGCGAGCAG	AA	ACTCAGC	AAGCGATCGA	540
25	CCC	GAGTGCA	CTGTTGTTCG	GCAGCGACAC	ACAGAAAGAC	GTCAACTTCG	GCACGCC	600	
	CAGCACC	CGTC	CAGAATCCGC	AGGACGCCAG	CAAGCC	CAACAGCAGT	CCAA	CATCGC	660
	TAAATTGATC	AGTGCATTGA	TCATGTC	TTG	GCTGCAGATG	CTCACCA	ACT	CCAATA	720
30	GCAGGACACC	AATCAGGAAC	AGCCTGATAG	CCAGG	CTCCT	TTCCAGAAC	ACGGC	GGGCT	780
	CGGTACACCG	TCGGCCGATA	GC	GGGGGGCGG	CGGTACACCG	GATGCGACAG	GTGGC	GGCGG	840
35	CGGTGATACG	CCAAGCGCAA	CAGGCGGTGG	CGGCGGTGAT	ACTCCGACCG	CAACAGGCGG	900		
	TGGCGGCAGC	GGTGGCGGCG	GCACACCCAC	TGCAACAGGT	GGCGGCAGCG	GTGGCACACC	960		
40	CACTGCAACA	GGCGGTGGCG	AGGGTGGCGT	AACACCGCAA	ATCACTCCGC	AGTTGGCAA	1020		
	CCCTAACCGT	ACCTCAGGTA	CTGGCTCGGT	GTGGACACC	GCAGGTTCTA	CCGAGCAAGC	1080		
	CGGCAAGATC	AATGTGGTGA	AAGACACC	AT	CAAGGTCGGC	GCTGGCGAAG	TCTTGACGG	1140	
45	CCACGGCGCA	ACCTTCACTG	CCGACAAATC	TATGGGTAAC	GGAGACCAGG	GCGAAAATCA	1200		
	GAAGCCC	CATG	TTCGAGCTGG	CTGAAGGC	TACGTTGAAG	AATGTGAACC	TGGGTGAGAA	1260	
50	CGAGGTCGAT	GGCATCCACG	TGAAAGCAA	AAACGCTCAG	GAAGTCACCA	TTGACAACGT	1320		
	GCATGCC	CAG	AACGTGGTG	AAGACCTGAT	TACGGTCAA	GGCGAGGGAG	GCGCAGCGGT	1380	
	CACTAATCTG	AA	CATCAAGA	ACAGCAGTGC	CAAAGGTGCA	GACGACAAGG	TTG	TCCAGCT	1440
55	CAACGCCAAC	ACTCA	TTGA	AAATCGACAA	CTTCAAGGCC	GACGATTTCG	GCACGATGGT	1500	
	TCGCACCAAC	GGTGGCAAGC	AGTTTGATGA	CATGAGCATC	GAGCTGAACG	GCATCGAAGC	1560		
	TAACCACGGC	AAGTTCGCCC	TGGTGAAAG	CGACAGTGAC	GATCTGAAGC	TGGCAACGGG	1620		

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CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680  
 CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAAGGG GGTGGACTC 1729

5 This DNA molecule is known as the *dspE* gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 14 as follows:

10	Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu 5 10 15
15	Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly 20 25 30
20	Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly 35 40 45
25	Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val 50 55 60
30	Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile 65 70 75 80
35	Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr 85 90 95
40	Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln 100 105 110
45	Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser 115 120 125
50	Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Asp Thr 130 135 140
	Pro Ser Ala Thr Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly 145 150 155 160
	Gly Gly Gly Ser Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly 165 170 175
	Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Val Thr 180 185 190
	Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr 195 200 205
	Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile 210 215 220
	Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp 225 230 235 240

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	Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp			
	245	250	255	
5	Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr			
	260	265	270	
	Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val			
	275	280	285	
10	Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln			
	290	295	300	
	Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala			
	305	310	315	320
15	Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp			
	325	330	335	
20	Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe			
	340	345	350	
	Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln			
	355	360	365	
25	Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly			
	370	375	380	
	Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr			
	385	390	395	400
30	Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln			
	405	410	415	
	Ala Ser Thr Gln His Thr Glu Leu			
35	420			

This protein or polypeptide is about 42.9 kDa.

40                  The hypersensitive response elicitor polypeptide or protein derived  
from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.  
ID. No. 15 as follows:

45	Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln			
	1	5	10	15
	Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser			
	20	25	30	
	Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile			
	35	40	45	
50	Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly			
	50	55	60	

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	Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala	
	65	70 75 80
	Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser	
	85	90 95
5	Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met	
	100	105 110
	Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala	
	115	120 125
	Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val	
10	130	135 140
	Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gln Gly Gly Leu Ala	
	145	150 155 160
	Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly	
	165	170 175
15	Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly	
	180	185 190
	Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Asn Gly Ala	
	195	200 205
	Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn	
20	210	215 220
	Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp	
	225	230 235 240
	Gln Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn	
	245	250 255
25	Ala Leu Val Gln Met Met Gln Gln Gly Leu Gly Gly Asn Gln	
	260	265 270
	Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly	
	275	280 285
	Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser	
30	290	295 300
	Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val	
	305	310 315 320
	Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln	
	325	330 335
35	Gln Ser Thr Ser Thr Gln Pro Met	
	340	

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 16 as follows:

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	ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCGG GTCTGCAGAA CCTGAACCTC	60
	AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC	120
	GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC	180
	GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC	240
5	AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC	300
	GGCAACGTCG ACGACGCCA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA	360
	GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG	420
	GGCAACGGCG TGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC	480
	GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCCGG TGCTGGCGCC	540
10	GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT	600
	GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC	660
	GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC	720
	CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG	780
	ATGATGCAGC AAGGCGGCCT CGGCGGCCGGC AACCAAGGCCG AGGGCGGCTC GAAGGGTGCC	840
15	GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CGGGCGCGA ACCAGCCCGG TTCGGCGGAT	900
	GATCAATCGT CGGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC	960
	GTCCAGATCC TGCAGCAGAT GCTGGCGGCCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG	1020
	ACCGCAGCCGA TGTAA	1035

20

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

25

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

30

Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Val	Ala	Ile	Ile	Ala	Ile	Leu	Ala	
1							5						10			15

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Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr  
20 25

5 This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

10 The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 18 as follows:

15	Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln	15
	5	
	1	
	Leu Leu Ala Met	
	20	

20 Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp N<sub>Ecc</sub>* and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," *MPMI*, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

25 30 Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and

35

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- Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A
- 5 Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.
- 10 Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.
- The above elicitors are exemplary. Other elicitors can be identified by
- 15 growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.
- Fragments of the above hypersensitive response elicitor polypeptides
- 20 or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the method of the present invention.
- Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then
- 25 are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.
- As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely
- 30 to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia*. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of suitable fragments of a hypersensitive response elicitor which do elicit a hypersensitive response are *Erwinia amylovora* fragments including a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the

following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.

Suitable DNA molecules are those that hybridize to the DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 2, 4, 5, 7, 9, 12, 13, and 16 under stringent conditions. An example of suitable high stringency conditions is when hybridization is carried out at 65°C for 20 hours in a medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate, 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 µm g/ml *E. coli* DNA.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the hypersensitive response elicitor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the hypersensitive response elicitor is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an

expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

5 U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation 10 and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

10 Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

15 Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which 20 is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced 25 into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

30 A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA;

microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promotor, *lac* promotor,

5      *trp* promotor, *recA* promotor, ribosomal RNA promotor, the  $P_R$  and  $P_L$  promotors of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promotor or other *E. coli* 10     promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

10     Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

15     Specific initiation signals are also required for efficient gene transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to 20     provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving 25     incorporation of synthetic nucleotides may be used.

30     Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention's method of imparting stress resistance to plants can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the elicitor to impart stress resistance. Alternatively, the hypersensitive response 5 elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart stress resistance in plants.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart stress resistance in plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. 10 When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart stress resistance to plants. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor 15 polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart stress resistance to plants.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated 20 hypersensitive response elicitor or 2) application of bacteria which do not cause disease and are transformed with genes encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding a hypersensitive response elicitor polypeptide 25 or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seed cells. In these embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide 30 variety of plants or their seeds to impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea,

chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable 5 ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

In accordance with the present invention, the term "stress" refers to drought, salt, cold temperatures (e.g., frost), chemical treatment (e.g., insecticides, fungicides, herbicides, fertilizers), water, excessive light, and insufficient light.

10 The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable 15 application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, 20 coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After 25 plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants.

30 The hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition 5 contains greater than 500 nM of the elicitor.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. 10 Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor can 15 be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed 20 with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by 25 reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be 30 accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby

incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies.

10. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

20. Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

25. 30. *Agrobacterium* is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a

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convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the

gene encoding the hypersensitive response elicitor resulting in stress resistance to the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart stress resistance to plants. While not wishing to be bound by theory, such stress resistance may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor in accordance with the present invention to impart stress resistance. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

## EXAMPLES

Example 1 - Hypersensitive Response Elicitor-Treated Cotton is More Resistant to the Damage Caused by Insecticide Stress

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Aphids (*Aphids gossypii*) infect cotton during the entire growth season. The damage of aphid infection ranges from honeydew deposit that contaminates the lint and reduces crop value to defoliation that reduces or destroys crops. To protect plants from aphid infection, cotton is usually sprayed with insecticides, for example Asana XL when the infection pressure is not very high, and Admire when the infestation pressure is high. The effect of a hypersensitive response elicitor on aphids in cotton was studied by a trial involving a randomized complete block design. This

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involved treatment with *Erwinia amylovora* hypersensitive response elicitor (i.e. HP-1000<sup>TM</sup>) at 20, 60, and 80 ppm and a chemical insecticide, Asana XL, at 8 oz./ac. Each treatment involved foliar application beginning at cotyledon to three true leaves and thereafter at 14 day intervals using a backpack sprayer. Aphid counts and overall growth of the cotton were made immediately prior to spray application at 14, 28, 35, and 42 days after the first treatment ("DAT 1"). Twenty-five randomly selected leaves per plot were collected at the first three sampling dates and the leaves per plot at the final sampling date.

## 10 Results

1. Aphid control: The number of aphids in the hypersensitive response elicitor-treated cotton were significantly reduced in comparison to the chemical treated cotton (see Table 1).

15 Table 1. Aphid count per leaf on cotton after treatment with Asana XL<sup>®</sup> or HP-1000<sup>TM</sup>

Treatment	Rate <sup>2</sup>	Number of aphids per leaf <sup>1</sup>			
		1/14DAT1	2/28DAT1	3/35DAT1	4/42DAT1
Asana XL <sup>®</sup>	8 oz/ac	0.2 a	32.2 a	110.0 a	546.9 a
HP-1000 <sup>TM</sup>	20 µg/ml	0.2 a	7.8 b	22.9 b	322.1 a
HP-1000 <sup>TM</sup>	60 µg/ml	0.1 a	4.9 b	34.6 b	168.3 a
HP-1000 <sup>TM</sup>	80 µg/ml	0.0 a	2.7 b	25.8 b	510.2 a

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05. <sup>2</sup>Rate for Asana XL<sup>®</sup> is for formulated product, rate for HP-1000<sup>TM</sup> is for active ingredient (a.i.).

20 At 14 days after DAT 1, aphid counts were relatively low across all of the treatments, but by 28 days after DAT 1 (by which time two sprayings had been applied), the number of aphids per leaf were significantly greater in Asana XL-treated plants compared to the hypersensitive response elicitor-treated cottons. By 35 days after DAT 1 (by which time three sprayings had been applied), aphid counts had risen for all treatments, yet aphid counts per leaf were still significantly lower for hypersensitive response elicitor-treated cotton compared to the Asana XL treatment. Finally, at 42 days after DAT 1 (by which time four sprayings had been applied), the number of aphids per leaf had increased to a level that threatened to overwhelm the

plants even when treated with the standard chemical insecticide. To save the trial, another chemical, Pravado (Admire), was applied to all plots to eradicate aphids from the field.

2. Hypersensitive response elicitor-treated cotton was more resistant to the damage caused by Pravado (Admire) and Asana. After the second chemical spraying, it was observed that cotton plants were stress shocked by the insecticides. The cotton plants previously treated with Asana and untreated control were defoliated. On most of the chemical-treated cotton, there were no leaves, or very few leaves, in the lower portion of plants. However, the hypersensitive response elicitor-treated plants, especially the plot where hypersensitive response elicitor was applied at 80 ppm, had no defoliation and the cotton plants were vigorous and healthy. By counting the number of mature balls, it clearly showed that hypersensitive response elicitor-treated plants (at 80 ppm) had more ball setting than chemical and untreated control (Table 2), indicating that hypersensitive response elicitor-treated plants were more tolerant to the stress caused by insecticide.

Table 2. Number of Formed Cotton Balls Counted on Ten Plants in Each of Four Replicates Per Treatment.

Treatment	<u>No. balls/10 plants/replicate</u>
UTC	28
Chemical standard	6
Hypersensitive Response Elicitor	35

Example 2 - Hypersensitive Response Elicitor-Treated Cucumbers are More Resistant to Drought

A cucumber field trial was set up to test the effect of *Erwinia amylovora* hypersensitive response elicitor on disease control, tolerance to drought stress, and yield. Three different rates were tested, there at 15, 30, and 60 µg/ml. In addition to hypersensitive response elicitor treatment, there was an untreated control. Each treatment contained three replicate plots. When the first true leaf emerges, the hypersensitive response elicitor was sprayed with a back bag sprayer. The second spray was applied ten days after the first spray. The third application was right after

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the recovery of cucumber seedlings after the transplanting to the field. Individual treatment was randomly assigned in the field.

When the first true leaf emerged (Day 0), a first application was sprayed. Usually cucumber seedlings are transplanted when seedlings show two true leaves. It has been known that the recovery rate after the transplanting is closely related to the size of the seedlings. Because of the drought, the seedlings were maintained in the nursery for an extra ten days and the second spray was applied on Day 10. Two days after the second spray, the plants were transplanted into fields and covered with plastic sheets. The plants had 4 – 5 true leaves.

10

### Result

The recovery rate of the transplanted cucumber seedlings was higher for the hypersensitive response elicitor-treated plants than for the untreated control. More than 80% of the hypersensitive response elicitor-treated cucumber seedlings survived, while only 57% untreated plants survived.

20

Throughout the growth season, there was a serious drought problem. Early field visits indicated that hypersensitive response elicitor-treated plants had more root mass and better over-all growth. Hypersensitive response elicitor-treated cucumber started to flower 14 days earlier than untreated control cucumber. The early flowering resulted in an earlier harvest. In the first harvest, more than 0.4 kilograms of cucumber fruits per plant were harvested from the hypersensitive response elicitor-treated cucumbers; however, virtually no fruit was harvested from untreated control. By the end of the season, untreated plants died due to severe drought, but hypersensitive response elicitor-treated plants were still alive and had one more harvest.

25

The final yield was significantly different between hypersensitive response elicitor-treated and untreated plants. Hypersensitive response elicitor administered at the rate of 30 ppm produced three times greater yield than the control plants (Table 3).

30

Table 3. Yield Increase of Cucumber Fruit from Hypersensitive Response Elicitor Treated Plants

Treatment	Replicate	kg/plant	Yield/Replicate	% of the Yield Increase
HP 15	I	1.25	37.5	
	II	1.00	30.0	103.8
	III	1.21	36.3	
HP 30	I	1.54	46.2	
	II	1.43	42.9	133.2
	III	1.47	44.1	
Control	I	0.43	12.9	
	II	0.41	12.3	39.3
	III	0.47	14.1	

5 The increased yield was partially attributed to hypersensitive response elicitor-induced growth enhancement and partially resulted from more tolerance of hypersensitive response elicitor-treated cucumber to drought, because usually the yield increase from hypersensitive response elicitor-induced growth enhancement is  
10 between 10-40%.

**Example 3 - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress**

15 Pepper seedlings were drenched with hypersensitive response elicitor at 20 ppm seven days before transplanting, sprayed seven days after the transplanting, and then, sprayed every fourteen days. Standard chemicals, Brave, Maneb, Kocide, and Admire, were used for the rest of the treatment. In addition to early growth enhancement, which resulted in a higher yield, larger fruit, and resistance to several diseases, hypersensitive response elicitor-treated pepper was more tolerant to  
20 diseases, hypersensitive response elicitor-treated pepper was more tolerant to herbicide damage. The pepper field was applied with the herbicide SENCOR which is not labeled for pepper. This herbicide is known to cause severe foliar damage to pepper in chemically-treated plants but not with hypersensitive response elicitor-treated plants.

25 The difference between the adverse effect of the herbicide on the hypersensitive response elicitor and non-hypersensitive response elicitor treated plants is dramatic. See Table 4 below. Thirty-nine of the 60 elicitor-treated plants showed only minor damage by the herbicide, the damaged leaves were less than 20%. In

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contrast, 53 out of the 60 chemically-treated pepper plants had severe damage, 40-57% of the leaves were damaged, and 20 plants were dead. The ability of hypersensitive response elicitors to help crops withstand the phytotoxic effects of a herbicide is very important benefit to in agricultural industry.

5

**Table 4. Hypersensitive Response Elicitor-Treated Peppers are More Tolerant to Herbicide Damage.**

	<u>Treatment</u>	<u>Damage Rating</u>						<u>Damage Index %</u>
		1	2	3	4	5	6	
10	Hypersensitive Response Elicitor	1	38	17	3	1	0	41
15	Chemicals	0	1	6	16	19	18	87

Damage Rating: 1. No damage; 2. 0-20% leaves damaged; 3. 20-40% leaves damaged; 4. 40-50% leaves damaged; 6. More than 75% leaves damaged or entire plant dead.

20 Damage index = sum of each rating times the number of plants under the rating scale, divided by total number of plants times 6.

Damage index for hypersensitive response elicitor-treated plants =  

$$\frac{1x1+2x38+3x17+4x3+5x1+6x0}{6} \times 100\% = 41\%$$

25

**Example 4 - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress under Controlled Experimental Conditions**

30 A field trial was conducted to test if hypersensitive elicitor treated pepper would be more tolerant to herbicide stress. The trial contains 6 treatments and 4 replicates for each treatment. The treatments are described as follows:

1. Control, the peppers were neither treated by a hypersensitive response ("HR") elicitor nor by LEXONE™ herbicide (DuPont Agricultural Products, Wilmington, Delaware).
2. Control pepper with application of 0.15 pound LEXONE™ herbicide /acre.
3. Control pepper with application of 0.3 pound LEXONE™ herbicide /acre.

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4. HR elicitor treatment with no application of LEXONE™ herbicide using a formulated product known as MESSENGER™ biopesticide (Eden Bioscience Corporation, Bothell, Washington) containing 3% HR elicitor protein was used.

5 5. HR elicitor treatment with application of 0.15 pound LEXONE™ herbicide /acre.

6. HR elicitor treatment with application of 0.3 pound LEXONE™ herbicide /acre.

10 LEXONE™ contains the same active ingredient as SENCOR™ herbicide (Bayer, Kansas City, Missouri) used in Example 3. Pepper seedlings were drenched with MESSENGER™ solution at the concentration of HR elicitor protein of about 20 ppm seven days before transplanting into the field and then sprayed every 14 days after the transplanting. LEXONE was applied at high (0.3 pound/acre) and low levels (0.15 pound/acre). 50 gallon water and 100 mL of the herbicide solution was introduced into the root zone of each plant in the respective treatment five weeks after transplant into the field.

15 The treatments were evaluated for the percent of chlorosis caused by the LEXONE™ herbicide application and for the pepper yield. HR elicitor-treated plants exposed to the high rate of herbicide had significantly less chlorosis and produced 108 % more fruit in comparison to the non-hypersensitive response elicitor treated plants exposed to the same amount of herbicide. See Tables 5 and 6 below. There was no significant difference in the reduction of chlorosis at the low rate of herbicide between the HR elicitor treated and non-HR elicitor treated peppers. However, the HR elicitor treated plants produced 15% more fruit than the corresponding control plants exposed to the same amount of herbicide. There was no chlorosis in either the check or HR elicitor-treated plants that did not receive LEXONE™ herbicide treatment.

20 The HR elicitor treated plants were much less severely affected by the herbicide application than the respective control plants at the high rate of herbicide. 30 However, the amount of visual chlorosis was similar at the low rate for both the check and HR elicitor-treated plants. More importantly, the yields from both the high and low rate herbicide treatments of HR elicitor treated plants were less severely effected

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by the herbicide than the checks. These findings further confirm that HR elicitors can help crops withstand the phytotoxic effects of herbicides and are very beneficial to the agricultural industry.

5      Table 5. Reduction of Foliar Chlorosis and Increase in Yield in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONE™ Herbicide

Treatment	Percent foliar chlorosis and yield of pepper					Yield (pound)	% difference from the respective control
	A	B	C	D	E		
6 (MESSENGER™ + High rate LEXONE™)	13.75	30.00	37.50	36.25	40.00	8.31	108 %
3 (High rate LEXONE™)	26.25	43.75	51.25	50.00	51.25	4.00	-
5 (MESSENGER™ + low rate LENOXE™)	16.25	22.50	28.75	23.75	27.50	8.00	15 %
2 (LENOXE™)	12.50	20.00	25.00	25.00	23.75	6.81	-

10      Table 6. Weight of Harvested Peppers Increased in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONE™ Herbicide Compared to Check Plants.

Treatment	Weight of peppers harvested 12/1/98 in pounds
HP20 + high rate LEXONE™	8.31
Check + high rate LEXONE™	4.00
HP20 + low rate LEXONE™	8.00
Check + low rate LEXONE™	6.81

15      Example 5 - Hypersensitive Response Elicitor-Treated Cotton is More Tolerant to Drought Stress

20      A non-irrigated cotton trial experienced 26 consecutive days of drought. The average daily heat index was near or over 100 degrees F, adding to the stress placed on the plants in the field.

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Observations in the field indicated that plants treated with HR elicitor at the concentration of 15 ppm (2.2 oz formulated product, MESSENGER™ containing 3 % active ingredient HR elicitor protein) were more vigorous and had less defoliation than the check plants as a result of the heat and drought stress. Equal numbers of plants from the MESSENGER™-treated and the non-MESSENGER™ treated plots were carefully removed from the field and mapped for the number of nodes and bolls by position. The plants were also weighed on a Metler analytical scale to determine whole plant, root and shoot weights.

MESSENGER™ treated plants survived the heat and drought stresses much better than the untreated plants did. Plants treated with MESSENGER™ had 37.6% more root and shoot mass than the check plants (Table 7). The MESSENGER™ treated plants also had significantly more cotton bolls than the check plants (Table 8). The number of cotton bolls from positions 1 and 2 have a significant contribution to the overall yield. Table 8 showed that MESSENGER™ treated plants had 47% more bolls in positions 1 and 2 and 57% more boll from a whole plant in comparison to the yield achieved using a grower standard treatment (i.e. with no MESSENGER™ treatment). A common reaction to stress in cotton is for the plant to abort bolls. The results indicate that MESSENGER™-treated plants are more tolerant to the drought stress.

20

Table 7. Weight per Plant of Non-Irrigated Cotton Following 26 Days of Drought.

Treatment	Root weight (pond/plant)	%Difference	Shoot weight (pond/plant)	% difference	Whole plant weight (pond/plant)	% difference
MESSENGER™ 2.2 oz/acre	0.041 a*	37.6 %	0.505 a	37.5 %	0.546	37.5 %
Control (Grower standard)	0.0298 b		0.367 b		0.397	
Level of statistically significant	P=0.119		P=0.034			P=0.033

25

\* Same letter indicates no statistical difference between the two treatments at the defined level; different letter indicates a statistical difference between the two treatments at the defined level.

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**Table 8.** Number of Bolls per 5 Plants at the Number 1 & 2 positions, and Total Number of Bolls from Whole Plants in Non-irrigated Cotton Following 26 days of drought.

5

Treatment	Avg. # bolls in the #1 & 2 position	Percent difference	Avg. # of total bolls per 5 plant	Percent difference
MESSENGER™ 2.2 OZ.	18.4 a	+46.0%	21.4 a	+57.0%
Check	12.6 b		13.6 b	-
Statistically significant level	P=0.032		P=0.01	

\* Same letter indicates no statistical difference between the two treatments at the defined level; different letter indicates a statistical difference between the two treatments at the defined level.

10      **Example 6 - Hypersensitive Response Elicitor-Treated Tomato is More Tolerant to Calcium Deficiency**

Calcium is an important element for plant physiology and development. A deficiency in calcium can cause several plant diseases. For example, 15 blossom-end rot is caused by a localized calcium deficiency in the distal end of the tomato fruit. Because calcium is not a highly mobile element, a deficiency can occur with a fluctuation in water supply. In the past, tomato growers experienced higher level of blossom-end rot during dry weather conditions when infrequent rains storms dumped a lot of water and then return to a hot and dry condition quickly. Lowering or 20 raising the irrigation water table erratically during a dry and hot growing season can also increase the disease.

A field trial was designed to test if HR elicitor protein-treated tomato can be more tolerant to the calcium deficiency under a dry hot growing season. MESSENGER™, the formulated product containing 3% HR elicitor, was used for the 25 trial. The application rate of the MESSENGER™ was 2.27 oz per care. The first spray of MESSENGER™ was carried out 7 days before the transplanting and then every 14-days after transplanting. MESSENGER™-treated tomatoes were compared with a standard grower treatment not utilizing MESSENGER™. Each treatment had 4 replicates.

30      The number of infected fruit was counted from a 100 square foot field. The rot typically begins with light tan water soaked lesions, which then enlarge, and then turn black. In a survey, about 20% of the fruits were infected. Severe end-rot

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symptoms occurred in the standard treatment; however, an average of only 2.5 % of the fruit was infected in the MESSENGER™-treated plants. The harvest data showed that MESSENGER™-treated plants had 8% more marketable fruit (Table 9). The test results demonstrated that MESSENGER™-treatment can reduce the stress resulting from calcium deficiency and increase plant resistance to blossom-end rot.

5

**Table 9. Hypersensitive Response Elicitor Treatment Reduced Blossom-End Rot Infection, Increased Yield of Tomato Fruit**

10

Treatment	Blossom-End Infected Fruit*				Tomato Fruit Yield	
	Rep I	Rep II	Rep III	Rep IV	Bin/Acre	% Difference
MESSENGER™	0	9	0	1	35	8
Standard Treatment)	24	22	16	17	31.5	-

\*The data were collected from the fruits in 100 square foot plot

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

15

**WHAT IS CLAIMED:**

1. A method of imparting stress resistance to plants comprising:  
applying a hypersensitive response elicitor protein or  
5 polypeptide in a non-infectious form to a plant or plant seed under conditions effective to impart stress resistance.
2. A method according to claim 1, wherein the stress resistance is  
resistance to a stress selected from the group consisting of climated related stress, air  
10 pollution stress, chemical stress, and nutritional stress.
3. A method according to claim 2, wherein the stress is chemical  
stress where the chemical is selected from the group consisting of insecticides,  
fungicides, herbicides, and heavy metals.  
15
4. A method according to claim 2, wherein the stress is climate-related stress selected from the group consisting of drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light.
- 20 5. A method according to claim 2, wherein the stress is air pollution stress selected from the group consisting of carbon dioxide, carbon monoxide, sulfur dioxide, NO<sub>x</sub>, hydrocarbons, ozone, ultraviolet radiation, and acidic rain.
- 25 6. A method according to claim 2, wherein the stress is nutritional stress where the nutritional stress is caused by fertilizer, micronutrients, or macronutrients.
- 30 7. A method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, or *Clavibacter*.

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8. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*, *Erwinia carotovora*, *Erwinia chrysanthemi*, and *Erwinia stewartii*.

5 9. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or  
10 *Pseudomonas solancearum*.

10. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Xanthomonas* species.

11. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Phytophthora*.

15 12. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Clavibacter michiganensis* subsp. *sepedonicus*.

13. A method according to claim 1, wherein plants are treated  
20 during said applying.

14. A method according to claim 1, wherein plant seeds are treated  
during said applying, said method further comprising:  
25 planting the seeds treated with the hypersensitive response  
elicitor protein or polypeptide in natural or artificial soil and  
propagating plants from seeds planted in soil.

15. A method according to claim 1, wherein the plant is selected  
from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower,  
30 peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel  
sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic,  
eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear,

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melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

16. A method according to claim 1, wherein the plant is selected  
5 from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

17. A method of imparting stress resistance to plants comprising:  
providing a transgenic plant or plant seed transformed with a  
10 DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and  
growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

15 18. A method according to claim 17, wherein a transgenic plant is provided.

19. A method according to claim 17, wherein a transgenic plant seed is provided, said method further comprising:  
20 planting the transgenic seeds in natural or artificial soil and propagating plants from seeds planted in soil..

20. A method according to claim 17, wherein the stress resistance is resistance to a stress selected from the group consisting of climatic related stress, air pollution stress, chemical stress, and nutritional stress.  
25

21. A method according to claim 20, wherein the stress is chemical stress where the chemical is selected from the group consisting of insecticides, fungicides, herbicides, and heavy metals.

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22. A method according to claim 20, wherein the stress is climate-related stress selected from the group consisting of drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light.

5 23. A method according to claim 20, wherein the stress is air pollution stress selected from the group consisting of carbon dioxide, carbon monoxide, sulfur dioxide, NO<sub>x</sub>, hydrocarbons, ozone, ultraviolet radiation, and acidic rain.

10 24. A method according to claim 20, wherein the stress is nutritional stress where the nutritional stress is caused by fertilizer, micronutrients, or macronutrients.

15 25. A method according to claim 20, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, or *Clavibacter*.

20 26. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*, *Erwinia carotovora*, *Erwinia chrysanthemi*, and *Erwinia stewartii*.

25 27. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or *Pseudomonas solancearum*.

28. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Xanthomonas* species.

30 29. A method according to claim 20, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic,

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eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

- 5           30.   A method according to claim 20, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

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## SEQUENCE LISTING

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RESISTANCE

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&lt;150&gt; 60/107,243

&lt;151&gt; 1998-11-05

&lt;160&gt; 18

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WO 00/28055

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&lt;210&gt; 8

&lt;211&gt; 1838

&lt;212&gt; PRT

&lt;213&gt; Erwinia amylovora

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Ser S r S r Ser Pr Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly  
 35 40 45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pr Ser Thr Ala Ala  
 50 55 60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg  
 65 70 75 80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln  
 85 90 95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala  
 100 105 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala  
 115 120 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met  
 130 135 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro  
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Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln  
 165 170 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp  
 180 185 190

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile  
 195 200 205

Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala  
 210 215 220

Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Ser Glu Phe Gln Gln  
 225 230 235 240

Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro  
 245 250 255

Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys  
 260 265 270

Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln  
 275 280 285



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Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val  
 290 295 300  
 Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro  
 305 310 315 320  
 Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys  
 325 330 335  
 Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln  
 340 345 350  
 His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr  
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 Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys  
 370 375 380  
 Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys  
 385 390 395 400  
 Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr  
 405 410 415  
 Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile  
 420 425 430  
 Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg  
 435 440 445  
 Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp  
 450 455 460  
 Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp  
 465 470 475 480  
 Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser  
 485 490 495  
 Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser  
 500 505 510  
 Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg  
 515 520 525  
 His Lys Met S r Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His  
 530 535 540



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Il Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His  
 545 550 555 560  
 Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Il Ser His Gly Arg  
 565 570 575  
 Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro  
 580 585 590  
 Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His  
 595 600 605  
 Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe  
 610 615 620  
 His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg  
 625 630 635 640  
 Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly  
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 Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His  
 660 665 670  
 His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly  
 675 680 685  
 Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr  
 690 695 700  
 Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly  
 705 710 715 720  
 Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu  
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 Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val  
 740 745 750  
 Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu  
 755 760 765  
 Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly  
 770 775 780  
 Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe  
 785 790 795 800



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Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu  
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 Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His  
 820 825 830  
 Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln  
 835 840 845  
 Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Trp His Lys  
 850 855 860  
 Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser  
 865 870 875 880  
 His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln  
 885 890 895  
 Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro  
 900 905 910  
 Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met  
 915 920 925  
 Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys  
 930 935 940  
 Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val  
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 Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr  
 965 970 975  
 Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His  
 980 985 990  
 Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly  
 995 1000 1005  
 Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro  
 1010 1015 1020  
 Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His  
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 Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu  
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 1075 1080 1085  
 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu  
 1090 1095 1100  
 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu  
 1110 1115 1120  
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 Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp  
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 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro  
 1140 1145 1150  
 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val  
 1155 1160 1165  
 Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser  
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 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe  
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 Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr  
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 Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp  
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 Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser  
 1265 1270 1275 1280  
 Met Ser Phe Ser Arg Ser Tyr Gly Gly Val Ser Thr Val Phe Val  
 1285 1290 1295  
 Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly  
 1300 1305 1310



WO 00/28055

Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly  
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Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Val Ser Gly Asn Ile  
 1330 1335 1340

Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys  
 1345 1350 1355 1360

Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile  
 1365 1370 1375

Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly  
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Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro  
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Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu  
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Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr  
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Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn  
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Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser  
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Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg  
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Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn  
 1490 1495 1500

Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala  
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Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly  
 1525 1530 1535

Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu  
 1540 1545 1550

Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu  
 1555 1560 1565



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Pro Val Thr Ser Asn Asp Il Ser Glu Leu Thr Ser Thr Leu Gly Lys  
 1570 1575 1580  
 His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu  
 1585 1590 1595 1600  
 Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His  
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 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg  
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 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser  
 1635 1640 1645  
 Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser  
 1650 1655 1660  
 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp  
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 Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Val  
 1730 1735 1740  
 Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser  
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 Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu  
 1765 1770 1775  
 Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile  
 1780 1785 1790  
 Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg  
 1795 1800 1805  
 Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser  
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gcgggtctgg aagtaccgca acacagcgac agcctgttac tacactgccc aatcatttag 180  
gctgaccac aaacttcaat aaccctgtat tcgatgttat tacagctgaa ttttcaaattg 240  
gcggccatgc gcccgttgtt gctggcgctg gatgaactgc acaacgtgcg ttatgtttt 300  
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35 40 45

Ser Asp Ser Leu Leu Leu His-Cys Arg Ile Ile Glu Ala Asp Pro Gln  
50 55 60

50  
Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met  
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65 Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val  
85 90 95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe  
 100 105 110

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu  
 115 120 125



WO 00/28055

Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala  
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<210> 11  
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 <212> PRT  
 <213> *Pseudomonas syringae*

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Ser Lys Ala Leu Gln Glu Val Val Lys Leu Ala Glu Glu Leu Met  
 35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Ala  
 50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Ile Glu Asp Val  
 65 70 75 80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
 85 90 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
 100 105 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
 180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly



WO 00/28055

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Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser			
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Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp			
245	250	255	
Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Leu Gly Thr Pro Val			
260	265	270	
Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln			
275	280	285	
Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Lys Gly Leu Glu Ala			
290	295	300	
Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala			
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Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg			
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&lt;210&gt; 12

&lt;211&gt; 1026

&lt;212&gt; DNA

<213> *Pseudomonas syringae*

<400> 12

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&lt;210&gt; 13

&lt;211&gt; 1729

&lt;212&gt; DNA

&lt;213&gt; Pseudomonas syringae

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&lt;210&gt; 14

&lt;211&gt; 424

&lt;212&gt; PRT

&lt;213&gt; Pseudomonas syringae



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 Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val  
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 Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile  
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                               115                                 120                                 125  
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                               145                                 150                                 155                                 160  
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                               165                                 170                                 175  
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 Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr  
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                               210                                 215                                 220  
 Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp  
                               225                                 230                                 235                                 240  
 Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp  
                               245                                 250                                 255



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 275 280 285  
 Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln  
 290 295 300  
 Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Ala Ala  
 305 310 315 320  
 Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp  
 325 330 335  
 Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe  
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 Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln  
 355 360 365  
 Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly  
 370 375 380  
 Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Leu Lys Leu Ala Thr  
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 <213> *Pseudomonas solanacearum*

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 Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile



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35

40

45

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 85 90 95

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Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
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Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
 130 135 140

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Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly  
 165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Asn Gly Ala  
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Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220

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Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Asn Gln  
 260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser



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290

295

300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
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 325 330 335  
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&lt;210&gt; 16

&lt;211&gt; 1035

&lt;212&gt; DNA

&lt;213&gt; Pseudomonas solanacearum

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&lt;210&gt; 17

&lt;211&gt; 26

&lt;212&gt; PRT

&lt;213&gt; Xanthomonas campestris pv. glycines

<400> 17  
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Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr  
 20 25



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&lt;210&gt; 18

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Xanthomonas campestris pv. pelargonii

&lt;400&gt; 18

Ser	Ser	Gln	Gln	Ser	Pro	Ser	Ala	Gly	Ser	Glu	Gln	Gln	Leu	Asp	Gln
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Leu Leu Ala Met

20



# INTERNATIONAL SEARCH REPORT

Inte Application No

PCT/US 99/26039

**A. CLASSIFICATION OF SUBJECT MATTER**  
**IPC 7 C12N15/82 C12N15/31 A01N63/02**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
**IPC 7 A01N**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 32844 A (CORNELL RES FOUNDATION INC) 30 July 1998 (1998-07-30) claims	1-30
A	WO 96 39802 A (CORNELL RES FOUNDATION INC) 19 December 1996 (1996-12-19) claims	1-30
A	WO 98 24297 A (CORNELL RES FOUNDATION INC) 11 June 1998 (1998-06-11) claims	1-30
A	WO 98 37752 A (CORNELL RES FOUNDATION INC) 3 September 1998 (1998-09-03) claims	1-30

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the International search	Date of mailing of the International search report
22 May 2000	07/06/2000
Name and mailing address of the ISA  European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Authorized officer  Decorte, D

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 99/26039

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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WO 9639802	A 19-12-1996	US 5650387 A AU 714512 B AU 5982196 A BR 9609073 A CA 2223616 A CN 1192647 A EP 0871354 A FI 974430 A JP 11506938 T NZ 309611 A PL 323823 A US 5859324 A US 5776889 A		22-07-1997 06-01-2000 30-12-1996 26-01-1999 19-12-1996 09-09-1998 21-10-1998 26-01-1998 22-06-1999 30-08-1999 27-04-1998 12-01-1999 07-07-1998
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WO 9837752	A 03-09-1998	AU 6666498 A BR 9807632 A US 5977060 A		18-09-1998 22-02-2000 02-11-1999